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①㉖ Anti-oxidants for use in the protection of easily oxidizable compounds, and a method for the protection of easily oxidizable compounds and application of the anti-oxidants.

①㉗ Anti-oxidants to protect easily oxidizable compounds in living and dead materials against oxidation, they are natural products, they are soluble in organic as well as aqueous solutions, they are preferably colourless and have no fluorescence, they have good flow properties, they have only faint smell and taste in the areas of concentration in question, and they contain primary and secondary amino-groups that are linked to carbon in aliphatic residue.

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The invention concerns anti-oxidants to be used for the protection of easily oxidizable compounds, especially polyunsaturated fatty acids, against oxidation. The anti-oxidants also have a growth-stimulating effect on fish, and may be used as an additive in feed.

Polyunsaturated marine fatty acids have attracted medical and nutritional attention in recent years because of their ability to protect against heart disease and cancer. It is supposed that the high incidence of these diseases is largely due to defective nutrition, and that the risk may be reduced by a change of eating habits or otherwise by consuming enough of the nutritionally important polyunsaturated fatty acids.

Polyunsaturated fatty acids and anti-oxidants are also of importance in the raising of domestic animals and in breeding. Fat is the cheapest source of energy in feed products, hence attempts are made to find diets containing a maximum of fats. Increasing the amount of fat does, however, increase the risk of rancidity. A balance must be found between the amount of fat it is desirable to add and the stability of the fat. In fish feed ethoxyquin is presently in use as an anti-oxidant. The compound is very effective, but is ~~not~~ allowed in foods for human consumption. It is worrying for Norwegian fish farms that ethoxyquin has been traced in edible fish. Alternatives to ethoxyquin are not yet available.

Anti-oxidants also prevent disease. For example, Norwegian fish farms lost between 100 and 200 million kroner in 1984, as a result of infection in farmed fish. It is supposed that the diseases are linked to poor feed quality, especially rancid fat.

The present invention takes as its starting point the marked reduction in the stability of fatty acids in the presence of acid. Acid is produced when fat turns rancid, which gives a positive feed-back leading to an acceleration of the rancidity process. I have postulated that there exists a type of anti-oxidant

which counteracts said process by neutralizing the acid. In order to fulfill their intended function, such anti-oxidants must have good fat-soluble properties, and they must be alkaline. Moreover, I have tried to find natural compounds of a known biochemistry.

Attention has been centred on polyamines. Said compounds occur in all living organisms, and have a simple structure of primary and secondary amino-groups which can neutralize acid. Polyamines also have physical and chemical properties that render them useful for a number of product types: they are fat and water-soluble, they flow easily, they have no marked taste or smell, and they are colourless.

The chemical structure of four natural polyamines is shown below:

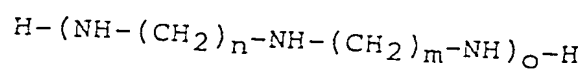
PUTRESCINE $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$

CADAVERINE $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$

SPERMIDINE $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$

SPERMINE $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2$

Thus, the invention concerns anti-oxidants which are to be used for protection of easily oxidizable compounds, especially polyunsaturated fatty acids, against oxidization, and which are naturally occurring compounds soluble in organic as well as aqueous solvents, and which are preferably colourless and have no fluorescence and only a faint smell and taste, said compounds being characterized in that they contain primary and secondary amino-groups which are linked to carbon in aliphatic residue and have the formula



where $n = 0-7$, $m = 0-7$ and $o = 1-4$ and where n and m can have different values for different values of o .

5 Specifically, the anti-oxidants according to the invention are spermine or spermidine.

10 Furthermore, the invention concerns a method for the protection of easily oxidizable compounds, especially polyunsaturated fatty acids, against oxidization, the method being characterized in that the compounds are mixed with the above anti-oxidants.

15 Furthermore, the invention concerns the application of the above anti-oxidants for oxidation protection of marine oils such as cod liver oil, capelin oil, and their concentrates, foodstuffs such as margarine, chocolate, dressings, meat and fish, feed products such as fish feed, feed for fur-bearing animals, poultry and domestic animals, cat and dog food, cosmetics such as lipstick, and skin creams, technical products such as lubricating oils, petrol, rubber and plastic, and pharmaceuticals.

20 The effect of different polyamines has been tested. Table 1 shows the effect of some common anti-oxidants on capelin oil (from *Mallotus villosus*) containing 7% easily oxidizable polyunsaturated fatty acids (20:5, 22:6). The stability was
25 investigated by means of an accelerated test, in which the oil was exposed to oxygen at a high temperature (100°C). The induction time, being the time from the start of the experiment until a rapid increase in a rancidity parameter takes place, is a measure
30 of the effect of an added anti-oxidant. The effects of different anti-oxidants given as % increase in induction time (protection factor) are shown in Table 1.

Table 1.

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Anti-oxidant 2mM		Protection factor
5	Blank	
	Vitamin E	0.0
	BHA	133.3
	Ascorbyl palmitate	142.9
	Ethoxyquin	723.8
10	Spermidine	1090.5
	Spermine	2947.6
		3957.1

15 The anti-oxidative effect of 200 ppm polyamines on a 30% concentrate of 20:5/22:6 is shown in Table 2. The peroxide development was monitored over a 60-day period. The mean temperature was 26°C.

20 The analyses were grouped in 7 series (A, B, C, D, E, F and G).

Series A

Samples were stored in a fridge (4°C), and samples were drawn from an unopened bottle once a week.

25 Series B

Samples were stored in the dark and at room temperature, and the material for analysis was taken from an unopened bottle once a week.

30 Series C

1 sample was stored in the dark in an open bottle at room temperature. Samples were taken 3 times a week.

The series D, E, F and G

35 The samples were stored in the dark in a sealed bottle and at room temperature. After sample taking, the bottles were agitated 5 times and sealed. Series D has no anti-oxidant, series E contains 200 ppm TBHQ (tertiary butyl hydroxy kinon), to series F has been

added 200 ppm octyl gallate. Series G contains 200 ppm spermine. The series C and D differ from each other in that C was open constantly, whereas D was opened 3 times a week and agitated. Measuring was performed 3 times a week.

5

Table 2

Series	Temp.	Conditions		Oxidation rate
		Open/sealed	Anti-oxidant	
10				
	A 4	SEALED		-0.027
	B 26	SEALED		-0.177
	C 26	OPEN		5.397
	D 26	OPEN/SEALED		3.597
15	E 26	OPEN/SEALED	TBHQ	3.414
	F 26	OPEN/SEALED	OCTYL GALLATE	3.210
	G 26	OPEN/SEALED	X	0.090

20

The investigation shows that spermine in open bottles gives the same protection as storage in sealed bottles. TBHQ and octyl gallate do not induce significant increases in stability.

25 The anti-oxidative effect depends both on the type of polyamine and on its concentration (Table 3). On a molar basis, the effect of putrescine is not as favourable as that of spermine. The variation in effect corresponds closely to the number of amino groups in the various polyamines the tests were performed on 30% concentrate of 20:5/22:6. Oxygen exposure at 70°C was used.

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Table 3.

A = spermine

B = spermidine

C = putrescine

	mM	Induction Time (hours)		
		A	B	C
10	0.0	369	351	354
	0.1	501	393	393
	0.5	1059	741	493
	1.0	1695	1158	663
	2.0	3156	2031	984

The anti-oxidative effect of polyamines has been confirmed through examining the fatty acid profile at different times during the oxidation process. Table 4 shows the fatty acid profile of capelin oil, stabilized by 0.8 mM spermine, at 0, 24.5 and 28.5 hours. The oil was oxidized at 100°C, and had an induction time of 24.5 hours.

Table 4.

Fatty acid	Composition after no. of hours			% variation from t = 0	
	0	24.5	28.5	t = 24.5	t = 28.5
18:4	1.6	1.5	0.7	-6.3	-56.3
20:5	4.0	4.0	1.3	0.0	-67.5
22:6	2.1	2.1	0.5	0.0	-76.2

Stabilisation of polyunsaturated fatty acids in fish feed is regarded as a key to increased growth, good general condition and reduced mortality.

The effect of spermine on char spawn is described below and in Table 5.

The results are:

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1. 200 ppm spermine in fish feed has no apparent negative effects on the fish.
2. Mortality is reduced from 4.5% in the monitor group to 1.1% in the pilot group.
3. The growth rate increases by 20% in the pilot group (Table 5).

It is concluded that polyamines may be used in fish feed.

- 15 The growth test was performed on 4 groups of char spawn. The total number of individuals was 332. The spawn was acclimatised over a 14-day period, during which time the temperature was increased gradually from 2.5 to 8.8°C. During the acclimatisation period the spawn was fed EWOS ST40-2.

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The feed given to each of the groups was:

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- A. Commercial EWOS ST40-2, stabilised with 200 ppm ethoxyquin.
- B. Freeze-dried capelin roe with added vitamins and minerals, but with no anti-oxidant.
- C. Freeze-dried capelin roe with added vitamins, minerals and 200 ppm ethoxyquin.
- D. Freeze-dried capelin roe with added vitamins, minerals and 200 ppm spermine.

Table 5

Day	0	14	28	42	56
Group	Wet weight (g)				
5					
A	0.980	1.897	2.468	2.761	3.016
B	0.980	1.726	2.025	2.083	2.285
C	0.980	1.702	2.068	2.111	2.307
D	0.980	1.593	2.115	2.205	2.804
10					
	% growth above monitor group (B)				
B	0.0	0.0	0.0	0.0	0.0
C	0.0	-1.4	2.1	1.3	1.0
15	D	0.0	-7.7	4.4	5.9
					22.7

Example 1

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Marine oils, such as capelin oil, cod liver oil, cod liver oil capsules and concentrates of polyunsaturated fatty acids are stabilised by adding the polyamines at a suitable stage of the production process. In the case of oils produced at low

25 temperatures, the anti-oxidants may be added before boiling. The concentration of the additive will depend on (a) what improvement in stabilisation is desired, and (b) what is the upper limit for anti-oxidants approved by the Health authorities.

30 Example 2

Foodstuffs such as margarine, chocolate and dressings are stabilised by adding the polyamines at a suitable stage of the production process. The anti-oxidants may be added either as an

35 aqueous solution or mixed with oil.

Example 3

Feed products such as fish feed and feed for fur-bearing animals are stabilised by adding the anti-oxidant at a suitable stage of the production process. The polyamines may be added to the raw materials (fish, fish offal, meat offal etc.) together with other water or fat-soluble additives.

Example 4

Cosmetics (both water and fat-based) may be stabilised by mixing in the polyamines at a suitable stage of the production process.

Example 5

Technological products such as petrol, lubricating oils, plastic, rubber and floating crystals are stabilised by mixing in the anti-oxidant at a suitable stage of the production process. In the case of polymer products, this may be before initiation is carried out.

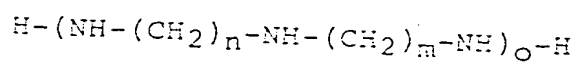
Example 6

Pharmaceuticals are stabilised by adding the polyamines in a concentration as stipulated by the Health Authorities. Polyamines may also be produced as separate pharmaceuticals and in suitable dispensary forms (tablets, retard tablets, solublets, pills, capsules, powders, granulates, mixtures, suppositories, injectabilia, infusion fluids, ampulles, dialytica, ointments, liniments, spray fluids etc.)

P a t e n t C l a i m s

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1. Anti-oxidants which are to be used for protection of easily oxidizable compounds, especially polyunsaturated fatty acids, against oxidation, and which are naturally occurring compounds
 5 soluble in organic as well as aqueous solvents, and which are preferably colourless and have no fluorescence, and which have only faint smell and taste, c h a r a c t e r i z e d i n
 that they are compounds containing primary and secondary amino groups which are linked to carbon in aliphatic residue and have
 10 the formula



15 where $n = 0-7$, $m = 0-7$ and $o = 1-4$ and where n and m can have different values for different values of o .

2. Anti-oxidants according to claim 1,
 c h a r a c t e r i z e d i n that they are spermine or
 20 spermidine.

3. Anti-oxidants according to claims 1 and 2,
 c h a r a c t e r i z e d i n that they are growth factors for
 fish.
 25

4. A method for the protection of easily oxidizable compounds, especially polyunsaturated fatty acids, against oxidization,
 c h a r a c t e r i z e d i n that the compounds are mixed with
 anti-oxidants according to claims 1 and 2.
 30

5. A method for increasing the growth rate in farmed fish,
 c h a r a c t e r i z e d i n that anti-oxidants are mixed into
 the feed according to claims 1 and 2.

35 6. A method according to claims 4 and 5,
 c h a r a c t e r i z e d i n that the anti-oxidants are used in
 concentrations of 2 - 1,000 ppm.

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7. Application of the anti-oxidants according to claims 1 and 2, for oxidation protection of marine oils, food stuffs, animal feed, cosmetics, technological products and pharmaceuticals.

8. Application of the anti-oxidants according to claims 1 and 2, for use as a pharmaceutical, a paramedical preparation and a cosmetic preparation.

9. Application of anti-oxidants according to claims 1 and 2 for use as a growth-inducing factor for animals and plants.

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(54) Anti-oxidants for use in the protection of easily oxidizable compounds, and a method for the protection of easily oxidizable compounds and application of the anti-oxidants.

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	CHEMICAL ABSTRACTS, vol. 104, no. 15, 14th April 1986, page 295, abstract no. 125403f, Columbus, Ohio, US; B. TADOLINI et al.: "Inhibition of lipid peroxidation by spermine bound to phospholipid vesicles", & BIOG. AMINES 1985, 3(2), 97-106 * Abstract *	1,2,4,6 ,7	C 09 K 15/18 A 23 K 1/1'6 C 11 B 5/00
X	CHEMICAL ABSTRACTS, vol. 33, no. 21, 10th November 1939, column 8799(2), Columbus, Ohio, US; E.A. EVANS et al.: "Effect of spermine on tissue oxidations", & PROC. SOC. EXPTL. BIOL. MED. 41, 467-70(1939) * Abstract *	1,2,4,6 -3	
X	FR-M- 3 517 (F. HOFFMANN-LA ROCHE) * Abstract I, points 1,4,5 *	1,2,4,6 -3	
X	US-A-3 031 485 (W. CANNON) * Claim 1 *	1,7	TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
X	US-A-4 507 321 (I. RAISFELD) * Claim 1; example 1 *	1,7	C 09 K A 23 K A 23 L C 11 B
A	FR-A-1 502 976 (MONSANTO) * Abstract I, no. (c) *	1,9	
X	FOOD, SCIENCE & TECHNOLOGY ABSTRACTS, abstract no. 86-05-j0086(86028684); A. ROVERSI: "Do polyamines enhance fruit-setting in sweet cherry?", & ANNALI DELLA FACOLTA DI AGRARIA, UNIVERSITA CATTOLICA DEL SACRO CUORE, vol. 23, no. 2, pages 201-206, 11 ref., 1983 * Abstract *	1,9	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 12-11-1987	Examiner DESMEDT G.R.A.
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			